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Short-interval amygdala kindling in neonatal rats

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Abstract

The kindling paradigm provides a powerful tool for studying the generation, propagation and generalization of seizures. Such reproducible quantitative paradigms are a prerequisite for the experimental study of epilepsy in the developing brain. Kindling has been extensively utilized as a model of limbic seizures in the adult rat; amygdala short-interval kindling has been studied in ≧15-day-old rats. We applied the short-interval kindling method, i.e., stimulation at every 15 min, to 7–12-day-old rats. Stage-5 behavioral seizures were achieved even in 7-day-old rats; however, the progression of behavioral kindling differed somewhat from that of older rats. Correlation of electrographic discharges and behavioral phenomena was inversely related to age. Reliable progressive amygdala discharges were difficult to assess in most ≦ 10-day-old rats. Spontaneous seizures occurred relatively frequently in younger age groups. The amygdala short-interval kindling paradigm is reproducibly and reliably applicable to rats during the 2nd postnatal week. The presence of progressive focal to bilateral-generalized seizures suggests a significant functional maturity of the amygdala-limbic circuitry at this age.

Keywords

Kindling; Neonatal; Amygdala; Rat; Seizure; Epilepsy model

INTRODUCTION

Since the seminal manuscript by Goddard[6], the kindling paradigm has proven immensely useful as a model of epilepsy and neuronal plasticity in adult rats (see Refs. 12 and 14 for reviews). Kindling-evoked after discharges (AD) may reflect the susceptibility of a conglomerate of neurons (focus) to stimulation[14,18]. The rate of progression of behavioral kindling stages from focal, through unilateral, to generalized phenomena provides a measure of both inhibitory and excitatory mechanisms in seizure propagation[12,14,18].

The amygdala kindling paradigm has been extensively utilized in > 15-day-old rats[7,9,11,19,20]. In these, unlike in adult rats[25], the “refractory period” is short[18,19]. They are thus readily capable of developing AD and generalized seizures following several hours of stimulation at 15-min intervals — the short-interval kindling (SIK) paradigm[7,18,19]. Moshe et al.[20] found the minimal current required for AD generation (AD threshold) to be higher in 15-day-old rats than in older ones. Only 75% of rats at that age could be kindled. Gilbert and Cain[5] concluded that 10- and 14-day-old rats could not be fully and reliably kindled.

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Fully kindled rats (subsequent to three or more stage-5 seizures) may develop spontaneous seizures[25]. The likelihood of such events increases with the number of stimulations[24]. The immature brain is widely considered more susceptible to a variety of convulsants and epileptogenic paradigms[3,15,18,19,30].

This study examines the response of 7–12-day-old rats to repeated amygdala stimulation at short 15-min intervals. The progressive behavioral and electrical kindling stages and the achievement of stage-5 seizures in rats starting on postnatal day 7 is demonstrated. Rate of kindling and the onset and incidence of spontaneous seizures are discussed.

MATERIALS AND METHODS

Animals

Timed-pregnant Sprague-Dawley-derived rats were obtained from Zivic-Miller (Zelionple, PA). They were housed under a 12-h light-dark cycle and fed ad libitum. Delivery times were monitored and were accurate to within 12 h. The day of birth was considered day 0. Pups were kept with the mothers and litters were culled to 12 individuals. They were subjected to surgery 24–48 h before kindling (24 h for < 9-day-old and 48 h for ≧ 9-day-old rats). Kindling was carried out starting between 09:00 and 09:30 to minimize the possibility of diurnal variations in seizure susceptibility[21].

Surgical procedure

Electrodes were implanted under halothane anesthesia, using an infant rat stereotaxic apparatus, as previously described[1,2,4]. Bipolar twisted wire electrodes, enameled except for the tip, were used. Electrodes (wire diameter 0.10–0.15 mm; vertical inter-tip distance 0.5–1.0 mm) were inserted through a burr-hole and aimed at the basolateral nucleus of the amygdala. Electrodes were anchored to the skull with an acrylic cement “cap” attached also to one or two screws[1–4]. Age-dependent basolateral amygdala coordinates, with reference to bregma, were adapted for our strain[22–27] (Table I).

Subsequent to each experiment, an electrolytic lesion (5–15 mA, 5–10 s) was generated and the electrode placement was verified. Animals were decapitated, brains were removed onto dry ice and blocked. Sequential 20-μm coronal sections were stained with cresyl violet.

SIK technique

Kindling paradigm was modified from Moshe et al.[19] and Haas et al.[7]. Briefly, kindling stimulus consisted of a 1-s train of a 60-Hz 400-μA peak-to-peak current, generated by a S10 SCM Grass stimulator. The latter was coupled to an isolation unit (GRASS SIU 8T) and a constant current unit (GRASS CCU1A), and visualized on a Tektronix 5111a oscilloscope. EEGs were obtained before and immediately after stimulations, which were delivered at 15-min intervals. Since 7–12-day-old rats displayed a unique sequence of kindling-induced behaviors, a modified[7,18] kindling scale was generated (Table II). Parameters measured were: the AD duration, the number of stimulations needed for the achievement of each kindling stage and the presence of “spontaneous” seizures. The latter were defined as stage-4 or -5 behaviors occurring, de novo, > 4 min after the most recent stimulation.

Rats with spontaneous seizures were usually stimulated once subsequent to the initial seizure to assess the length of the refractory period between the seizure and subsequent stimulation. They were then observed for 1–2 h. All pups with “spontaneous” seizures continued to manifest them intermittently until sacrifice.

Analysis

Of 51 7–12-day-old rats, correct placement of electrodes was achieved in 41: nine (7-day-old), nine (8-day-old), 10 (9-day-old), five (10-day-old) and eight rats (12-day-old). Only rats with correct placement were included in the analysis. They were combined into three age groups: 7–8-day-old ($n = 18$), 9–10-day-old ($n = 15$) and 12-day-old rats ($n = 8$). Pooled data are presented as $x \pm$S.E.M. Individual data points for each rat (stimulation number and behavioral stage) are displayed (Fig. 2) to demonstrate the inter-animal variability. The significance of difference between age groups was analysed using the Mann–Whitney rank sum test.

RESULTS

The spectrum of progressive stimulation-induced behaviors was age-dependent. In 7–9-day-old rats, as in 10–12-day-old ones, behavior arrest and head/face movements were observed initially. Subsequent stages in the youngest age group (7–9-day-old rats) consisted of tonic neck flexion or forelimb rotation, and only later did forelimb clonus follow (Table II). Further, alternating clonus, prominent in 10–12-day-old and older rats[7,18], was infrequent.

Inter-animal variability was higher in 7–9-day-old rats than in older ones: the number of stimulations required for each behavioral stage differed substantially (Fig. 2). Overall, 7–8-day-old rats progressed faster to stage 3 (4.94 ± 0.5 stimulations; $n = 18$) than 10–12-day-old ones (5.7 ± 0.6 stimulations; $n = 23$). Kindling rate of the younger age group to stage-4 behavioral kindling was higher: a mean of 8.2 stimulations in 7–8-day-old rats vs. 11.5 stimulations in 12-day-old rats ($P < 0.005$).

Using a constant "supra-threshold"[20] stimulus current of 400 μA resulted in discernible AD in all age groups (Fig. 1). AD were consistently present in $\geqq$ 9-day-old rats (Fig. 1b–d) but only in 9/18 7–8-day-old rats (Fig. 1a). In some rats, rhythmic respiration-movement artifacts[2] and poorly defined AD contour precluded positive identification of electrographic discharges. 9-day-old rats tended to have a longer AD duration at each stimulation than 12-day-old ones (Fig. 3).

Spontaneous seizures were observed with an earlier onset and a higher incidence in the youngest rats (Table III). The incidence of such seizures was 50% in 7–8-day-old rats. They occurred subsequent to kindling stages 3.5–5 and persisted until sacrifice (2–3 h).

DISCUSSION

Kindling may be a measure of susceptibility to seizure generation[6,12,14]. The paradigm thus provides a quantitative, reproducible experimental model. The classic kindling paradigms, requiring daily (or every 12 h) stimulations for weeks, are not applicable to the study of rapidly developing immature rats. Rapid, or short-interval, kindling permits examination of seizure susceptibility at a precise age[7–9,11,15,18–20].

Both amygdala[7,9,11,19,20] and hippocampal[8,15,] rapid kindling paradigms have been well characterized in "suckling" or "weanling" rats (> 15-day-old). Haas et al.[7] devised a behavioral scale to account for the different progression of stimulation-induced seizures in the immature brain. Amygdala kindling has been used to assess the effect of anatomical, metabolic and hormonal inputs on seizure susceptibility in the immature rat[9,11,18].

Amygdala kindling in < 15-day-old rats has not been reported. Moshe et al.[20] found an increased AD threshold in 15- vs. 35-day-old rats; 25% of the younger age group could not be kindled. We utilized a current of 400 μA, well above the 237 ± 13 μA AD threshold

determined by these investigators. Gilbert and Cain[5] were unable to induce amygdala kindling in 10-day-old rats and only a "weak stage 3" response in 14-day-old ones. The authors used kindling parameters similar to ours but applied stimuli at 2-h intervals which may account for their results.

We were unable to induce bilateral generalized seizures in all 7–8-day-old rats. Such variability, also observed in the rate of kindling at this age (Fig. 2), has been noticed by others. Lack of sustainable cortical discharges during the first 10 days of life may partially account for this observation[2,3,13].

Stage-5 behavioral seizures (body extension and loss of balance) occur in 7–8-day-old rats more rapidly than in older ones. Furthermore, spontaneous seizures occur frequently (in up to 50% of the rats). This provides evidence for the functional maturity of excitatory limbic circuits of seizure propagation in 7–12-day-old rats. Recent evidence[10,16,26] suggests the involvement of excitatory amino-acid receptors, specifically glutamate receptor, in kindling. Ontogenic studies[23] confirm the high-level expression of non-NMDA glutamate receptor genes in rat limbic structures during the first postnatal week. Moreover, the ratio of glutamate receptor-subunit prevalence differs in neonatal and adult rats[23] which may result in a different pattern of Ca^{2+} permeability and postsynaptic currents[29]. This, in conjunction with increased prevalence of the prolonged Ca^{2+} -promoting "flip" form of receptor units[17], may account for the increased incidence of "spontaneous", kindling-associated seizures observed in neonatal rats. Similar predilection of preadolescent amygdala-kindled kittens for spontaneous seizure development has been reported[28].

In conclusion, this study reports on the successful amygdala SIK in 7–12-day-old rats, when brain development roughly corresponds to that in the newborn human. This experimental paradigm should thus permit further study of mechanisms of epilepsy development during this critical and highly vulnerable age.

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References

1. Baram TZ, Hirsch E, Snead OC III, Schultz L. CRH induced seizures in the infant brain originate in the amygdala. Ann Neurol. 1992; 31:488–494. [PubMed: 1596084]
2. Baram TZ, Hirsch E. EEG recording in neonatal rats: some pitfalls and solutions. Dendron. 1992; 1:39–46.
3. Baram TZ, Snead OC III. The ontogeny of bicuculline induced seizures in infant rats-behavioral and electrocortical phenomena. Dev Brain Res. 1990; 57:291–295. [PubMed: 2073726]
4. Baram TZ, Schultz L. CRH is a rapid and potent convulsant in the infant rat. Dev Brain Res. 1991; 61:97–101. [PubMed: 1914160]
5. Gilbert ME, Cain DP. A developmental study of kindling in the rat. Dev Brain Res. 1982; 2:321–328.
6. Goddard GV, McIntyre D, Leech CK. A permanent change in brain function resulting from daily electrical stimulation. Exp Neurol. 1969; 25:295–330. [PubMed: 4981856]
7. Haas KZ, Sperber EF, Moshe SL. Kindling in developing animals: expression of severe seizures and enhanced development of bilateral foci. Dev Brain Res. 1990; 56:275–280. [PubMed: 2261687]
8. Holmes GL, Thompson JL. Rapid kindling in the prepubescent rat. Brain Res. 1987; 433:381–384.
9. Holmes GL, Weber DA. Effects of ACTH on seizure susceptibility in the developing brain. Ann Neurol. 1986; 20:82–88. [PubMed: 3017186]

10. Holmes KH, Bilkey DK, Laverty R, Goddard GV. The *N*-methyl-D-aspartate antagonists aminophosphonovalerate and carboxypiperazinephosphonate retard the development and expression of kindled seizures. Brain Res. 1990; 506:227–235. [PubMed: 1967965]
11. Lee SS, Murata R, Matsuura S. Effects of Hypoglycemia on kindling seizures in suckling rats. Exp Neurol. 1988; 99:142–153. [PubMed: 3275549]
12. Lothman EW, Bertram EH III, Stringer JL. Kindling, a model of epilepsy, in: functional anatomy of hippocampal seizures. Prog Neurobiol. 1991; 37:1–82. [PubMed: 1947175]
13. Mares J, Mares P, Trojan S. The ontogenesis of cortical self-sustained after-discharges in rats. Epilepsia. 1980; 21:111–121. [PubMed: 7353563]
14. McNamara JO, Byrne MC, Dashieff RM, Fitz JG. The kindling model of epilepsy: a review. Prog Neurobiol. 1980; 15:139–159. [PubMed: 6109361]
15. Michelson HB, Lothman EW. An ontogenic study of kindling using rapidly recurring hippocampal seizures. Dev Brain Res. 1991; 61:79–85. [PubMed: 1914159]
16. Mody I, Heinemann U. NMDA receptors of dentate gyrus granule cells participate in synaptic transmission following kindling. Nature (London). 1991; 349:760–765. [PubMed: 1847995]
17. Monyer H, Seeberg PH, Wisden W. Glutamate-operated channels: developmentally early and mature forms arise by alternative splicing. Neuron. 1991; 6:799–810. [PubMed: 1673851]
18. Moshe, SL. The ontogeny of seizures and substantia nigra modulation. In: Kellaway, P.; Noebels, JL., editors. Problems and Concepts in Developmental Neurophysiology. Johns Hopkins Press; Baltimore, MD: 1989. p. 247-261.
19. Moshe SL, Albala BJ, Ackerman RF, Engel J Jr. Increased seizure susceptibility of the immature brain. Dev Brain Res. 1983; 7:81–85.
20. Moshe SL, Sharpless NS, Kaplan J. Kindling in developing rats: variability of afterdischarge thresholds with age. Brain Res. 1981; 211:190–195. [PubMed: 7225835]
21. Oliverio A, Castellano C, Puglisi S, Renzi P. Diurnal variation in electroconvulsive shock induced seizures: involvement of endogenous opioids. Neurosci Lett. 1985; 57:237–240. [PubMed: 4041021]
22. Paxinos, G.; Tork, I.; Tecott, LH.; Valentino, KL. Atlas of the Developing Rat Brain. Academic Press; San Diego, CA: 1991.
23. Pellegrini-Giampietro DE, Bennet MVL, Zukin RS. Differential expression of 3 glutamate receptor genes in developing rat brain: an in situ hybridization study. Proc Natl Acad Sci USA. 1991; 88:4157–4. [PubMed: 1851996]
24. Pinel JP, Rovnar LI. Electrode placement and kindling-induced experimental epilepsy. Exp Neurol. 1978; 58:335–346. [PubMed: 618751]
25. Racine RJ, Burnham WM, Gartner JG. First trial motor seizures triggered by amygdaloid stimulation in the rat. Electroencephal Clin Neurophysiol. 1973; 35:487–494.
26. Sato K, Morimoto K, Okamoto M. Anticonvulsant action of a non-competitive antagonist of NMDA receptors (MK-801) in the kindling model of epilepsy. Brain Res. 1988; 463:12–20. [PubMed: 2848606]
27. Sherwood, NM.; Timiras, PS. A Stereotaxic Atlas of the Developing Rat Brain. University of California Press; Berkeley, CA: 1970.
28. Shouse MN, Langer J, Dittes PR. Spontaneous sleep epilepsy in amygdala kindled kittens: a preliminary report. Brain Res. 1990; 525:215–224. [PubMed: 2253028]
29. Verdoon TA, Burnashev N, Monyer H, Seeberg PH, Sakmann B. Structural determinants of ion flow through recombinant glutamate receptor channels. Science. 1991; 252:1715–1718. [PubMed: 1710829]
30. Vernadakis A, Woodbury DM. The developing animal as a model. Epilepsia. 1969; 10:163–178. [PubMed: 4308259]

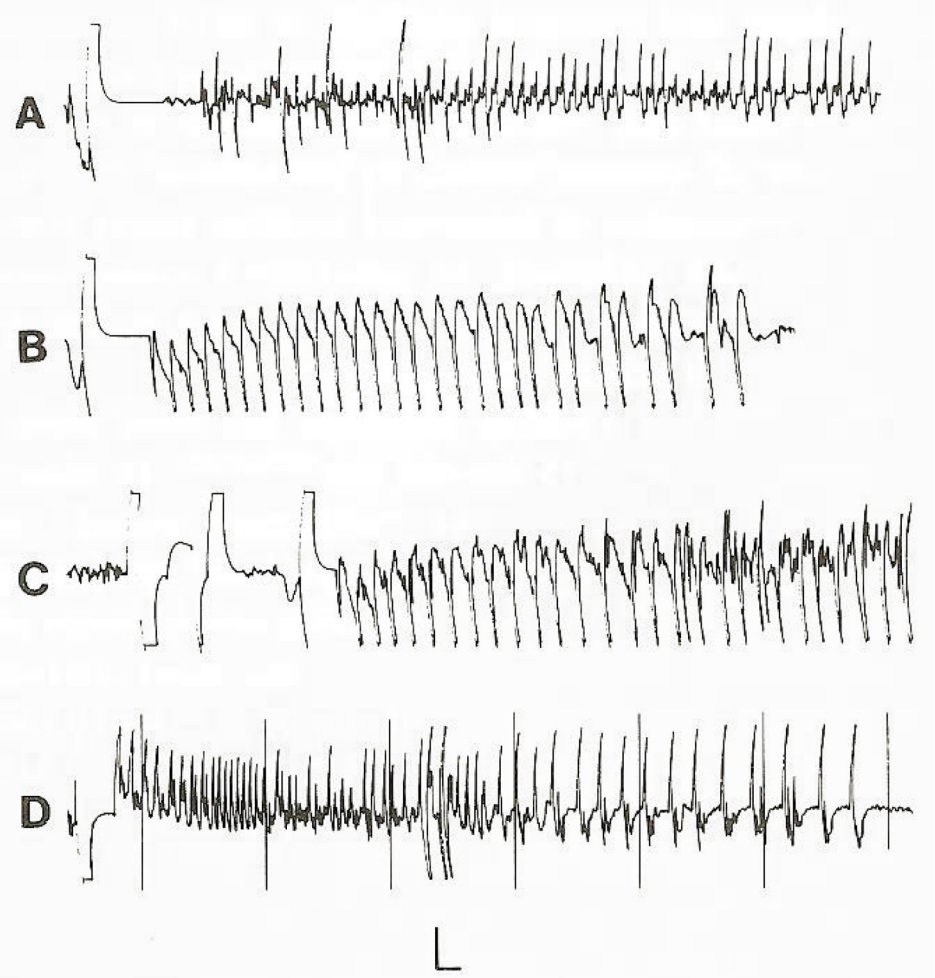

Fig. 1.

AD in 7–12-day-old rats subjected to short-interval stimulation. A: 7-day-old rat, subsequent to fifth stimulation. B: 9-day-old rat, fifth stimulation. C: 10-day-old rat, 19th stimulation. D: 12-day-old rat, 17th stimulation. Horizontal bar, 1 s; vertical bar, 10 μV in A, 20 μV in B and C, and 40 μV in D.

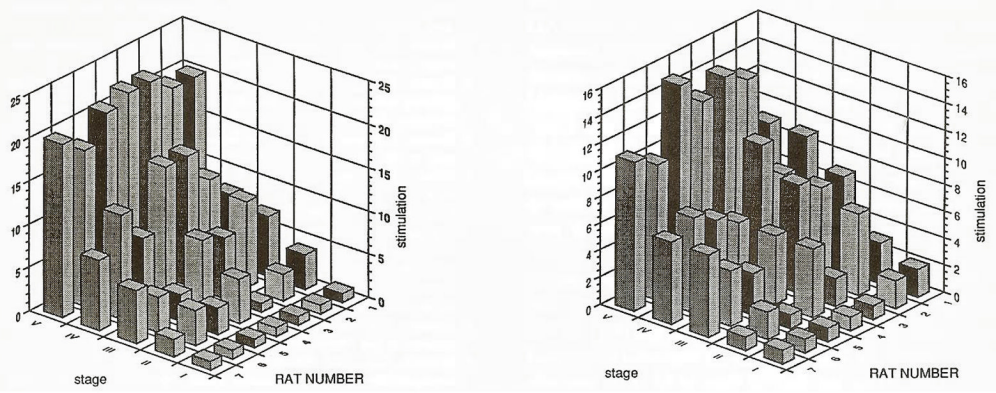

Fig. 2.

Comparison of kindling rates in 7–8- and 12-day-old rats. Individual rats are plotted, showing number of stimulations for each behavioral stage. Inter-individual variability and kindling rate are higher in younger rats. Note different y axes (stimulation number).

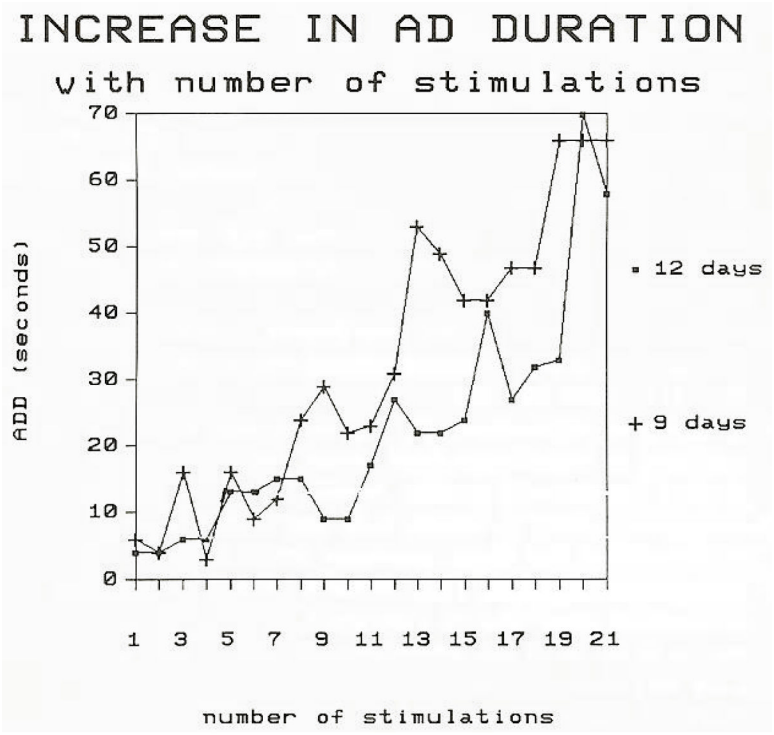

Fig. 3.

Correlation of AD duration and stimulation number in 9- and 12-day-old rats. See text for details of kindling paradigm.

TABLE I

Coordinates for electrode placement

Measurements are bregma. Animals are placed in infant stereotaxic apparatus with skull horizontal between bregma and lambda.

AGE (days)	AP	LAT	V
5–6	−1.5	3.5	6.0
7–9	−1.5	3.6	8.0
10–12	−1.5	3.6	8.0
13–14	−1.5	3.6	8.3
15–17	−1.5	3.6	8.5

TABLE II

Scale for kindling-induced behaviors in neonatal rats

Stage	Days 7–9	Days 11–12
0	Behavior arrest	Behavior arrest
1	Head bob/facial movement	Head bob/facial movement
2	“Chewing”/neck flexion	Neck flexion, “chewing”
3	Vigorous lick/limb rotation	Unilateral clonus/body flexion
3.5	Unilateral clonus *	Alternating clonus
4	Rearing (rare)	fp + rotation/bilat clonus/rear
5	Tonic extension	Loss of balance/extension

* Alternating clonus seen rarely in 7-day-old rats.

+ fp, forepaw.

TABLE III

"Spontaneous" seizures during kindling in neonatal rats

Age (days)	Number of rats with seizures (%)	Median stimulation number at onset (range)
7–8	9/18 (50)	11 (5–12)
9–10	7/14 (50)	17 (15–19)
12	2/8 (25)	19, 19